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ON FURTHER ANTIGEN RELATIONS BETWEEN PASTEURELLA PSEUDO-TUBERCULOSIS AND THE SALMONELLA GROUP

/Following is a translation of an article by W. Knapp of the Hygiene Institute of Tubingen University in the German-language periodical Zeitschrift fur Hygiene (Journal of Hygiene), No 146, 1960, pages 315-330./

The antigen relations between Past. pseudo-tuberculosis type II and the Salmonella B sub-group and between Past. pseudo-tuberculosis type IV (following Thal) and the Salmonella D sub-group were first demonstrated by Schutze (1928/1932) and Knapp (1955) respectively.

Kauffmann (1932) discovered, in the given antigen relation of Past. pseudo-tuberculosis to O-factor 4 of the Salmonella B sub-group, the complex nature 4_1 and 4_2; whereas Knapp (1955) disclosed the complex nature 9_1 and 9_2 of the O-factor 9 of the Salmonella D sub-group by demonstrating the antigen relations between Past. pseudo-tuberculosis type IV and the O-factor 9 of the Salmonella D sub-group. The findings have been confirmed by several authors (citations in Knapp, 1959).

With the aid of investigation results summarized in various tables, the demonstration of additional antigen relations between Past. pseudo-tuberculosis and the Salmonella group will be reported.

I. Further Antigen Relations Between Past. pseudo-tuberculosis Type II and the Salmonella B Sub-group.

Crosswise absorption and agglutination experiments were conducted with various Pasteurella and Salmonella sera.

These normally involved 4 to 5 intravenous injections of increasing amounts of antigen at intervals of 4 to 5 days and, when necessary, a booster injection after 2 to 3 months.

The following summary gives the designation of the Pasteurella and Salmonella strains used to immunize the guinea pigs and the manner of killing the cultures incubated either for 48 hours at 22 degrees C or for 24 hours at 37 degrees C and always rinsed with a 10 ml physiological salt solution:

Serum No 26: Past. pseudotuberculosis strain 16; serological type II, subtype A (16 II A); killed with 0.5% phenol.

Serum No 467: Past. pseudotuberculosis strain 16 II A; boiled 2-½ hours.

Serum No 279: Past. pseudotuberculosis strain 1779; serological type II, subtype B (1779 II B); heated 2-½ hours at 56 degrees C.

Serum No 273: Past. pseudotuberculosis strain 1779 II B; boiled 2-1/2 hours.

Subtype A and subtype B sera of Past. pseudotuberculosis type II were produced by saturating sera Nos 26 and 467 with Past. pseudotuberculosis strain 1779 II B (lbd) and sera Nos. 273 and 279 with Past. pseudotuberculosis strain 16 II A (lbd). (See Table 1)

The cross-reactions resulting from the partial antigen community of Past. pseudotuberculosis type II and O factor 4 of the Salmonella B sub-group were eliminated by rinsing sera Nos. 26, 467, 273, and 279 with S. Abortus equi (strain 202) and S. reading (strain 19). See Table 1)

Serum No 125: S. abortus bovis 1960 (1, 4, 12, 27); boiled 2-½ hours.

Serum No 131: S. schwarzengrund; strain 150 (1, 4, 12, 27); boiled 2-½ hours. In the O-factor 27 sera, resulting from saturation of sera 125 and 131 with S. reading and S. abortus equi, the agglutinin titer stood at 1:80 as against O-factor 1. (See Table 1)

The evaluation of test results summarized in Table 1 led to the following conclusions:

1. The agglutination in varying degrees of boiled suspensions of S. abortus equi (4, 12) and S. reading (4, 12) in the sera 26 and 467 or sera 279 and 273, produced with Past. pseudotuberculosis strain 16 II A or 1779 II B, is conditioned by the known antigen relation between Past. pseudotuberculosis type II and O-factor 4 of the Salmonella B sub-group. (For details see Schurtze, 1928/1932; Kauffmann, 1932; Knapp, 1959.)

2. The agglutination of boiled suspensions of S. schleissheim 13, S. schwarzengrund, and S. abortus equi in the Past. pseudotuberculosis sera 26 (16 II A) and 279 (1779 II B) saturated with S. reading and S. abortus equi, as in reserve from Past. pseudotuberculosis 16 II A and 1779 II B (very weak agglutination) in the Salmonella sera 125 (S. abortus bovis) and 131 (S. schwarzengrund) saturated with S. reading and S. abortus equi up to O-factor 27 sera, rests on an antigen relations between Past. pseudotuberculosis type II and O factor 27 of the Salmonella B sub-group, [see Note]. This antigen relation appears to depend on the presence of a thermolabile antigen, for only in sera 26 and 279, but not in sera 467 and 273 produced with boiled suspensions, were agglutinin evident in connection with O-factor 27.

(Note. With thanks to Prof. Kauffmann of the State Serum Institute, Copenhagen, for his kindness in checking these results.)

3. According to the results of the agglutination reactions summarized in Table 1, O-factor 27 is, like O-factor 4 of the Salmonella B sub-group, of complex nature. A partial antigen appears to have antigen relations with the specific antigen type of Past. pseudotuberculosis type II. The indications of this are: a) that after saturation of serum 26 (16 II A) with S. schleissheim or with S. schleissheim and S. reading, only the homologous strain (16 II A) and not the heterologous strain (1779 II B) agglutinated; and that after corresponding saturation of serum 279 (1779 II B), only strain 1779 II B and not strain 16 II A were agglutinated; and b) that in the sub-type specific sera of Past. pseudotuberculosis type II A and type II B, S. schleissheim, S. schwarzengrund, and S. abortus bovis were not agglutinated.

4. With Past. pseudotuberculosis strain 16 II A, which was agglutinated in the O-factor sera 125 and 131 up to 1:60 serum dilution, and with Past. pseudotuberculosis strain 1779 II B and others of the same sub-type, which were not agglutinated or were agglutinated up to a titer of 1:80 visible only in the agglutinoscope, no complete saturation of O-factor 27 sera was successful. These strains led only to further saturation of varying degrees of the Salmonella sera 125 and 131 originally saturated with S. reading and S. abortus equi.

5. The observation that, after saturation of O-factor sera 125 and 131 with Past. pseudotuberculosis strain 16 II or with the weakly agglutinable strain 1779 II B, the strains 1779 II B and 16 II A were not agglutinated, suggests the existence of an antigen relation between the specific antigen type of Past. pseudotuberculosis type II (of complex nature) and O-factor 27 of the Salmonella B sub-group. This partial antigen appears in our experiments to be less strongly developed by chance in strain 1779 II B than in 16 II A, except as it is altogether weakly developed in this strain.

With S. reading and S. abortus equi, the type-specific but no subtype-specific agglutinins were removed from the Pasteurella sera 467 and 273 produced with boiled antigens. The saturated sera 467 and 273 agglutinated only the homologous strains 16 II A and 1779 II B. But in the sera 26 and 279, produced with killed antigens, both strains (16 II A and 1779 II B) were agglutinated after their preparation with S. reading and S. abortus equi, while only the homologous strains agglutinated after saturation with S. schleissheim.

The differing findings, probably traceable to the varying preparation of the antigens used for immunization (see Table 1), indicate at least that Past. pseudotuberculosis type II possesses a thermostable and thermolabile type-specific antigen. The thermostable antigen shows partial affinity with O-factor 4, the thermolabile antigen with O-factor 27 of the Salmonella B sub-group.

Further investigations of the antigen communities between Past. pseudotuberculosis and the Salmonella group disclosed weak antigen

relations between Past. pseudotuberculosis strain 32 type IV and O-factor 14 of the Salmonella H sub-group. These observations, confirmed by Kauffmann as slight antigen relations between Past. pseudotuberculosis and S. carrau 34 /Note: See Kauffmann (1958) for Kauffmann-White scheme), will be treated at the end of this study. But in further tests Kaufmann found a strong agglutination of Past. pseudotuberculosis strain 32 IV in an O-factor 46 Salmonella serum, not then available to us, and, in reverse, of S. strassburg (/9/ 46:d:1.7) in a serum produced with Past. pseudotuberculosis strain 32 IV. These observations, kindly communicated by Prof. Kauffmann by letter (1958), were confirmed by our further tests with various type IV strains. They are used here with his consent.

II. Further Antigen Relations Between Past. pseudotuberculosis Type IV and the Salmonella D_2 Sub-group

The following guinea pig immunization sera (and others not presented in detail) were tested in alternating absorption and agglutination experiments for their agglutinin content and the results summarized in Table 2:

Serum No. 120: Past. pseudotuberculosis strain 32; serological type IV (32 IV); boiled 2½ hours.

Serum No. 196: Past. pseudotuberculosis strain 190; serological type IV (190 IV); boiled 2½ hours.

Sera No. 462 and 459: S. strassburg; Salmonella Central, Bonn; (9:46); boiled 2½ hours.

The cross-reactions resulting from the partial antigen association between Past. pseudotuberculosis type IV and O-factor 9 of Salmonella D_1 and D_2 sub-groups were eliminated through the saturation of Sera 462 and 459 with S. typhi O 901 (Ibd) or S. gallinarum 416 (Ibc). According to Kauffmann (1958), S. strassburg has partial antigen association with O-factor 10 of Salmonella E_1 sub-group, so that an O-factor 46 serum is obtained by saturating a S. strassburg O-serum with S. enteritidis (or S. typhi) and S. london. Since cross-agglutinations showed that Past. pseudotuberculosis strains 32 IV and 190 IV (Saisawa and Ikegachi) are not agglutinated in an O-factor 10 serum, and that S. london is not agglutinated in sera of the four named Pasteurella strains, we had to be content with the saturation of sera 459 and 462 with S. typhi or S. gallinarum. (See Table 2.)

The evaluation of test results summarized in Table 2 led to the following conclusions: 1. The agglutination of boiled suspensions of S. typhi O 901 W or S. gallinarum 416 in Sera 120 and 196 produced with Past pseudotuberculosis strains 32 IV and 190 IV is conditioned by the known partial antigen association between Past. pseudotuberculosis type IV and O-factor 9 of the Salmonella D sub-group. (For details see Knapp, 1955, 1959; Toucas and Girard, 1956.)

2. The agglutination of boiled suspensions of S. strassburg in Pasteurella sera 120 and 196 saturated with S. typhi or S. gallinarum, as in reverse with Past. pseudotuberculosis strains 32 IV and 190 IV in O serum 459 saturated with S. typhi, results from an antigen relations between Past. pseudotuberculosis type IV and O-factor 46 of the Salmonella D_2 sub-group. Contrary to expectations, however, an antigen relation was shown not to exist in serum 462 saturated with S. gallinarum in relation to O-factor 46, although S. strassburg was agglutinated up to 1:160 serum dilution.

3. Since Pasteurella sera 120 and 196 could not be fully saturated either with S. strassburg alone or in combination with S. typhi, likewise O-serum 459 neither with Past. pseudotuberculosis strain 32 IV alone nor in combination with S. typhi, the O-factor 46 of Salmonella D_2 sub-group and the type-specific antigen of Past. pseudotuberculosis type IV must be of complex nature. Past. pseudotuberculosis type IV thus has, among others, two type-specific stable partial antigens standing in antigen association with O-factor 9 or 46 of Salmonella D_1 and D_2 sub-groups (in $2\frac{1}{2}$ hour boiling).

According to experiments by Uetake and Nakano (1949), the strains of Past. pseudotuberculosis found in a soldier by Saisawa (1909) and in monkeys by Kawashima (1934) and/or Ikegaki (1936) have antigen relations with Salmonella D sub-group. Since data on the types of the strains were lacking, it has been unknown whether the two strains known as Ikegaki and Saisawa also belong to type IV, corresponding to the division of types by Thal (1954). According to Thal, the two available strains 32 IV and 190 IV are distinguished from types I, II, III, and V by their O and H antigens, for the others, while differing as to the O antigen, have the same H antigen. (Citations in Knapp, 1959)

After Dr. Yamad of Japan kindly provided us with the two strains shortly before the end of our experiments and they revealed differences in antigen structure as compared with the strains 32 IV and 190 IV available to us, the following additional questions arose:

1. Do the strains Ikegaki and Saisawa belong to type IV and can subtypes be distinguished in type IV as in types I and II of Past. pseudotuberculosis?

2. Does the antigen relation, demonstrated by Uetake and Nakano (1949), to the Salmonella D sub-group also rest on a partial antigen association with O-factor 9 and 46 or only with one of the two O-factors?

3. Do the two strains have antigen relations with Past. pseudo-, tuberculosis of types I, II, III, and V?

For the necessary crosswise saturation and agglutination tests to answer the first two questions, the following sera were newly produced or utilized. The answer to the third question occurs in another place.

Serum No. 744 and 787: Past. pseudotuberculosis strain Ikegaki; boiled 2½ hours.

Serum No. 766 and 770: Past. pseudotuberculosis strain Saisawa; boiled 2½ hours.

Serum No. 120 and 196: Past. pseudotuberculosis strains 32 IV and 190 IV. (See Table 2).

Table 3 shows: 1. The O antigen structures of Past. pseudotuberculosis strains Ikegaki and Saisawa are not identical. After saturation of the sera 744 and 787, produced with the strain Ikegaki, with strain Saisawa, the strain Ikegaki was agglutinated up to 1:160 and/or 1:320 serum dilution; whereas on the other hand in the sera produced with strain Saisawa and saturated with strain Ikegaki, serum 776 was not agglutinated and serum 770 was weakly agglutinated -- visible only in the agglutinoscope -- up to 1:80 serum dilution. The question raised by the observations, whether strains Ikegaki and Saisawa have different subtype-specific antigens, in the case of Saisawa temporarily not or only weakly developed, could not be answered by the experiments to date.

2. Differences in the antigen structure between Past. pseudotuberculosis strains 32 IV and 190 IV on the one hand and strains Ikegaki and Saisawa on the other result from the presence of at least one subtype-specific antigen.

The immunization sera 774, 787, 766, and 770 (See Table 3), produced with boiled suspensions of strains Ikegaki and Saisawa, agglutinated in varying degrees the strains 32 IV and 190 IV, without being fully saturated with 32 IV; just as the sera 120 and 196 obtained from strains 32 IV and 190 IV (See Table 2) agglutinated the strains Ikegaki and Saisawa without being fully saturated with them.

According to these observations, type IV of Past. pseudotuberculosis can at least be divided between subtypes A and B.

3. Like Past. pseudotuberculosis strains 32 IV and 190 IV, the Ikegaki and Saisawa strains have antigen relations to O-factors 9 and 46 of the Salmonella D_1 and D_2 sub-groups. The demonstration of partial antigen association with O-factor 46 succeeded, however, only in a serum with a high agglutinin titer.

a) S. typhi and S. strassburg were agglutinated in the unsaturated and S. strassburg also in the S. typhisaturated Pasteurella sera 744, 787, 766, and 770, though with varying strength; whereas the Ikegaki and Saisawa strains were agglutinated in various S. typhi O-sera and in the S-typhi-saturated serum 459 up to 1:160 and/or 1:60 serum dilution, but not in the correspondingly produced and saturated serum 462, although the serum saturated with S. gallinarum agglutinated S. strassburg up to 1:160 of se um dilution. (See Table 2).

b) Only in serum 459, which is produced like serum 462 with S. strassburg (2½ hours at 100 degrees) and agglutinated S. strassburg after saturation with S. typhi up to a titer of 1:1280, were the strains 32 IV, 190 IV, Ibagaki, and Saisawa agglutinated. Agglutination was absent, however, with these strains in serum 462, which agglutinated S. strassburg after saturation with S. Gallinarum only up to 1:160 dilution.

4. The results of cross-wise saturation and agglutination experiments among the four strains of type IV and between them and S. strassburg cannot be related in detail. But it appears certain that a) the type-specific antigen of Past. pseudotuberculosis type IV is of complex nature and the antigen relation among the various strains of type IVA and IVB does not exist only through the type-specific partial antigen common to O-factors 9 and 46 of the Salmonella D_1 and D_2 sub-groups; and b) Past. pseudotuberculosis strains of type IV show differences in their type-specific partial antigens, which may however be of quantitative rather than qualitative sort.

This conclusion rests on the following observations: a) after the saturation of sera 744 and 787, made from strains Ibagaki and Saisawa, and/or sera 766 and 770 with strain 32 IV or S. strassburg, for Ibagaki and Saisawa only subtype-specific agglutinins remained; thus, the Salmonella D_1 and D_2 sub-groups must have been removed by strain 32 IV and/or S. strassburg (see Table 3). But after saturation of these sera with S. typhi there were still antibodies relating to S. strasburg (O-factor 46) remaining, which, however, led to no agglutination of Past. pseudotuberculosis strains 32 IV and 190 IV even though these strains have antigen relations to O-factor 46. One can surmise there from that the agglutination of 32 IV and 190 IV was absent because of a temporarily too weak development of these partial antigens in strains Ibagaki and Saisawa, or because the agglutinogeneous impulse of the partial antigens was too weak, so that only the serologically closely related strains Ibagaki and Saisawa were affected by the antibodies of saturated sera except for S. strassburg. It is also pertinent that strains Ibagaki and Saisawa have a further partial antigen in common with S. strassburg.

b) The saturation of sera 120 and 196, produced with the serologically identical strains 32 IV and 190IV, with strain Ibagaki (see Table 2) led to the elimination of the agglutinins common to strains Ibagaki and Saisawa, as well as to serum 120 and S. typhi, but not to serum 196. These were, however, in serum 196 considerably saturated with the Saisawa strain. In both sera, as expected, the antibodies corresponding to O-factors 9 and 46 were eliminated with S. strassburg, and those corresponding to O-factor 9 with S. typhi. After saturation with S. strassburg, agglutinins for Past. pseudotuberculosis strains 32 IV and 190 IV remained with high titers, those for Ibagaki and Saisawa strains with low titers, while S. strassburg, as well as the Past. pseudotuberculosis strains, was agglutinated after preparation of the sera with S. typhi.

While S. typhi and S. strassburg, in the sera 744, 787, 766, and 770 produced with Ikegaki and Saisawa strains, led (contrary to expectations) to a saturation of the antibodies agglutinating strains 32 IV and 190 IV, in the reverse case after corresponding saturation of sera 120 and 196, produced with strains 32 IV and 190 IV, antibodies for strains Ikegaki and Saisawa remained in varying titers. This observation seems to us to support the previously mentioned supposition that, beyond the partial antigens common to O-factors 9 and 46 of the Salmonella D sub-group, still other probably type-specific partial antigen associations exist between individual strains of type IV, whereby the individual partial antigens may be developed in varying strengths.

The antigen relations observed in the course of these experiments of Ikegaki and Saisawa strains to, e.g., Past. pseudotuberculosis type I, probably resulting from the presence of a common thermolabile antigen, require further clarification.

III. Antigen Relations Between Past. pseudotuberculosis Type IV and the Salmonella E Subgroup

Cross-wise saturation and absorption tests were conducted with the following three sera and others mentioned in the text, the findings being summarized so far as necessary in Table 4.

Serum No 196: Past. pseudotuberculosis strain 190 IV; boiled 2½ hours (see page 320).

Serum No 120: Past. pseudotuberculosis strain 32 IV; boiled 2½ hours.

Serum No 595: S. boecker; strain 245, EBZ (6, 14, lv:1.7); living.

Serum No 272: S. boecker; strain 245, EBZ (6, 14); boiled 2½ hours.

O-factor 14 sera were obtained by saturating sera 595 and 272 with S. thompson 8 (6, 7, k, 1.5) and S. potsdam 9781 (6, 7, lv, enx).

In another immunization serum produced with S. boecker no agglutination for O-factor 14 could be ascertained after corresponding saturation. The production of suitable sera, comprehending the common partial antigen, with strains of Past. pseudotuberculosis type IV and of Salmonella E subgroup involved great difficulty. The immunization of guinea pigs was repeatedly unsuccessful.

Our several tests, only partly presented in Table 4, show that a weak antigen relation prevails among the four available type IV strains and the O-factor 14 of the Salmonella E subgroup:

1. In sera 196 and 120, produced with strains 190 IV and 32 IV, S. carrau, S. boecker, S. madalia 611, and S. onderstepoort 282 (not shown in Table 4) were agglutinated up to a serum dilution ranging from 1:160 to 1:320. S. potsdam 9781 and S. thompson 8 were not agglutinated in these sera; likewise, the four Past. pseudotuberculosis strains of

type IV in O-factor 24 and 25 sera. Strains 190 IV and 32 IV, on the other hand, were agglutinated in the full serum 595, saturated with S. potsdam and S. thompson, up to 1:160; also strain Ikegaki to 1:80 and strain Saisawa weakly to 1:40 serum dilution. A complete saturation of Pasteurella sera 196 and 120 with S. carrau or S. boecker was as unsuccessful as that of O-factor 14 serum with strain 32 IV and the Ikegaki strain. These findings show that an admittedly weak partial antigen association exists between Past. pseudotuberculosis type IV and O-factor 14 of the Salmonella H subgroup, as confirmed by Kauffmann.

2. But in the immunization sera produced with strain Ikegaki (sera 787 and 744) and with strain Saisawa (sera 770 and 766), S. carrau and S. boecker were not agglutinated. Since the Ikegaki and Saisawa strains were agglutinated only to 1:80 and 1:40 serum dilution in unsaturated and with S. thompson and S. potsdam saturated serum 595, the partial antigen association between these two type IV strains and the O-factor 14 appears to be very slight.

3. The findings for serum 272 (S. boecker, 2½ hours) corresponded to those for serum 595 and require no tabular presentation.

Summary

Besides the already known antigen relations between Past. pseudotuberculosis and the Salmonella group, partial antigen associations exist between Past. pseudotuberculosis type II and O-factor 27 of the Salmonella B subgroup, and between Past. pseudotuberculosis type IV and the O-factors 46 and 14 of the Salmonella subgroups D_2 and H, though the connection with the H subgroup is weakly developed.

In Past. pseudotuberculosis of type IV, subtypes A and B were identified. Strains 32 IV and 190 IV belong to subtype A, while subtype B includes the previously untyped Ikegaki and Saisawa strains, the serological behavior of which is not clarified in all details.

The results of the various test series were discussed at the end of the corresponding sections.

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Table 1. Cross Reactions between Past. pseudotuberculosis Type II and Salmonella B Sub-group

Serum No.	Saturated with	Antigens for agglutination (2½ hrs. 100 degrees)						
		Past. pseudotb		Salmonella				
		16 II (A)	1779 II (E)	schleissheim (4, 12, 27)	schwarzengrund (1, 4, 12, 27)	abortus (1, 4, 12, 27)	bovis abortus (4, 12)	reading (4, 12)
26 P. Ps.tb strain 16 II (0.3% phenol)	without	640	1280	5120	1280	1280	320	1280
	Past. ps.tb 1779 II lbd	160	—	—	—	—	—	—
	S. reading + S. ab. equi lbd[2]	320	160	160	80	—	—	—
	S. schleissheim lbd	320	—	—	—	—	—	—
	S. schleissheim + S. reading lbd	320	—	—	—	—	—	—
467 P. ps.tb strain 16 II (2½ hrs.100°)	without	2560	320	320	160	320	1280	—[1]
	Past. ps.tb 1779 II lbd	1280	—	—	—	—	—	—
	S. reading + S. abortus equi lbd	320	—	—	—	—	—	—
	S. schleissheim lbd	160	—	—	—	—	—	—
	S. schleissheim + S. abortus equi lbd	160	—	—	—	—	—	—
279 P. ps.tb strain 1779 II (2½ hrs.56°)	without	320	1280	640	320	80	160	640
	Past. ps.tb 16 II lbd	—	160	—	—	—	—	—
	S. reading lbd	160	640	160	80	—[1]	—	—
	S. schleissheim lbd	—	320	—	—	—	—	—
	S. schleissheim + S. reading lbd	—	640	—	—	—	—	—

[Table 1, cont.]

273 P. ps.tb strain 1779 II (2½ hrs.100°)	without	160	1280	320	1280	80	640	320
	Past. ps.tb 16 II 1bd	—	640	—	--	--	--	—
	S. reading 1bd	—	320	—	—	—	—	—
	S. schleissheim	—	320	—	--	--	—	—
	S. schleissheim + S. reading 1bd	—	320	—	—	—	—	—
125 S. abortus bovis (2½ hrs.100°)	without	640	1280	1280	640	5120	640	160
	S. reading + S. ab. equi 1bd	160	—3	320	160	1280	—	—
	S. reading + S. ab. equi + Past. ps.tb 1779 II 1bd	—	—	80	160	160	—	—
	S. reading + S. ab. equi + Past. ps.tb 16 II 1bd	—	—	160	160	320	—	—
131 S. schwarzengrund (2½ hrs.100°)	without	640	1280	640	5120	640	5120	5120
	S. reading + S. ab. equi 1bd	160	—1	160	640	160	—	—
	S. reading + S. ab. equi + Past. ps.tb 1779 II 1bd	—	—	80	640	160	—	—
	S. reading + S. ab. equi + Past. ps.tb 16 II 1bd	—	—	160	320	80	—	—

1. Very weak agglutination to serum dilution 1:40 or 1:80, visible only in agglutinoscope.

2. In serum 18 (Past. ps. tb strain 16 II 1bd), S. schleissheim and S. schwarzengrund were agglutinated, after saturation with S. reading and S. ab. equi, only to 1:80 and 1:40 serum dilution.

3. Serum 242 (Past. ps. tb 1779 II, 2½ hrs. 100°) showed the same results after saturation with S. ab. equi and S. reading.

Table 2. Cross Reactions between Past. pseudotuberculosis Type IV and Salmonella D_1 and D_2 Sub-groups

Serum No.	Saturated With	Antigens for agglutination (24 hrs. 100 degrees)						
		Past. pseudotb		Salmonella			Past. pseudotb	
		32 IV	190 IV	strassburg (9,46)	typhi (9, 12)	gallinarum (1, 9,	Ikegaki	Saisawa
120 P. Ps. tb Strain 32 IV (24 hrs. 100 degrees)	without	2560	320	5120	320	2560	320	160
	S. typhi lbd	1280	320	640	—	—	320	160
	S. strassburg lbd	1280	320	—	—	—	80	80
	S. strassburg + S. typhi lbd	1280	320	—	—	—	—	—
	Past. ps. tb 190 IV lbd	1280	640	160	—	—	—	—
	Past. ps. tb Ikegaki lbd	320	160	—	—	—	—	—
	Past. ps. tb Saisawa lbd							
196 Past ps. tb Strain 190 IV (24 hrs. 100 degrees)	without	2560	2560	1280	640	640	640	320
	S. typhil lbd	2560	1280	320	—	—	160	—
	S. strassburg lbd	2560	2560	—	—	—	160	80
	S. strassburg + S. typhil lbd	1280	320	—	—	—	—	—
	Past. ps. tb 32 IV lbd	2560	640	80	160	— 1	—	—
	Past. ps. tb Ikegaki lbd	1280	640	— 1	— 1	— 1	—	—
	Past. ps. tb Saisawa lbd							

[Table 2, cont.]

459 S. strassourg (2½ hrs. 100 degrees)	without	640	320	5120	320	1280	320	80
	S. tyohi lod	160	160	1280	—	—	160	80
	Past. ns. tb 32 IV lbd	—	—	640	—	—	160	80
	Past. ns. tb 32 IV 100°	—	—	320	—	—	80	80
	S. tyohi +							
	Past ns. tb 32 IV lbd	—	—	640	—	—	1	1
	Past. ns. tb Ikegaki lbd	—	—	320	—	—	—	—
	Past. ns. tb Saisawa lbd	—	—	320	—	—	—	—
462 S. strassburg (2½ hrs. 100 degrees)	without	320	160	5120	160	80	160	160
	S. gallinarum lbd	—	—	160	—	—	—	—
	Past ns. tb 32 IV lbd	—	—	320	—	—	80	80
	Past ns. tb 32 IV 100°	—	—	160	—	—	—	—
	S. gallinarum +							
	Past ns. tb 32 IV lbd	—	—	160	—	—	—	—
	Past ns. tb Ikegaki lbd	—	—	160	—	—	—	—
	Past ns. tb Saisawa lbd	—	—	160	—	—	—	—

1. Very weak agglutination to serum dilution 1:40 or 1:80, visible only in agglutinoscope.

Table 3. Cross Reactions between Past. pseudotuberculosis Strain Ikegaki and Saisawa and Past. pseudotuberculosis Strain 32 IV and 190 IV and O-Factors 9 and 46 of the Salmonella D_1 and D_2 Sub-groups

Serum No.	Saturated with	Antigens for agglutination (2½ hrs. 100 degrees)					
		Past. pseudotb				Salmonella	
		32 IV	190 IV	Ikegaki	Sai-sawa	strass-burg	typhi
784 Past. ps. tb Strain Ikegaki (2½ hrs. 100 degrees)	without	160	160	1280	320	320	80
	S. typhi lbd	—	—	160	80	320	—
	S. strassburg lbd	—	—	320	320	—	—
	Strain Saisawa lbd	—	—	160	—	—	—
	Strain Saisawa 100°	—	—	160	—	—	—
	Past. ps. tb 32 IV lbd	—	—	320	160	—	—
787 Past. ps. tb Strain Ikegaki (2½ hrs. 100 degrees)	without	1280	320	5120	1280	320	160
	S. typhi lbd	—	—	320	160	160	—
	S. strassburg lbd	—	—	320	160	—	—
	Strain Saisawa lbd	—	—	320	—	—	—
	Strain Saisawa 100°	—	—	320	—	—	—
	Past. ps. tb 32 IV lbd	—	—	640	160	—	—
766 Past. ps. tb Strain Saisawa (2½ hrs. 100 degrees)	without	640	160	5120	640	80	160
	S. typhi lbd	—	—	160	320	80	—
	S. strassburg lbd	—	—	320	320	—	—
	Strain Ikegaki lbd	—	—	—	—	—	—
	Strain Ikegaki 100°	—	—	320	160	—	—
	Past. ps. tb 32 IV lbd	—	—			—	—

[Table 3, cont.]

770 Past. os. tb Strain Saisma (24 hrs. 100 degrees)	without	320	160	1280	320	640	160
	S. typhi lbd	—	—	320	160	80	—
	S. strassburg lbd	—	—	160	80	—	—
	Strain Ikegaki lbd	—	—	—	—[1]	—	—
	Strain Ikegaki 100°	—	—	—	—	—	—
	Past. os. tb 32 IV lbd	—	—	320	160	—[1]	—

1. Very weak agglutination to serum dilution 1:40 or 1:80, visible only in agglutinoscope.

Table 4. Cross Reactions between Past. pseudotuberculosis and O-factor 14 of the Salmonella H Sub-group

Serum No	Saturated with	Antigens for agglutination (2½ hrs. 100 degrees)							
		Past. pseudotb				Salmonella			
		32 IV	190 IV	Ikegaki	Saisawa	carrau (6, 14, 24)	boecker (6, 14)	potsdam (6, 7)	thompson (6, 7)
196 Past. ps. tb Strain 190 IV (2½ hrs. 100 degrees)	without	2560	2560	640	320	160	160		
	S. carrau lbd	1280	640	320	160	—	—	2	—
	S. boecker lbd	2560	2560	640	320				
	S. typhi lbd	1280	640	160	80	160	80		
	Past ps. tb strain Ikegaki lbd	2560	640	—	—	1	1		
	Past ps. tb strain Saisawa lbd	1280	640	—	—	1	1		
120[3] Past. ps. tb Strain 32 IV (2½ hrs. 100 degrees)	without	2560	320	640	320	160	160		
	S. carrau lbd	1280	160	160	80	—	—		—
	S. boecker lbd	640	320	160	80				
	S. typhi lbd	1280	320	320	160	80	160		
	Past. ps. tb strain Ikegaki lbd	1280	320	—	—	80	80		
	Past ps. tb strain Saisawa lbd	320	160	—	—	1	1		
595 S. boecker lbd	without	160	160	80	1	320	1280	80	160
	S. potsdam + S. thompson lbd	160	160	80	1	320	320		
	Past. ps. tb strain 32 IV	—	—	—	—	160	320	80	160
	Past. ps. tb strain Ikegaki lbd	—	—	—	—	320	640	1	160

[Table 4, cont.]

1. Very weak agglutination to serum dilution 1:40 or 1:80, visible only in agglutinoscope.

2. ./. signified not tested

3. With another serum (No. 847) produced with strain 32 II, S. carrau showed no agglutination; S. agglutination up to 1:80 and 1:160 serum dilution.